Michigan Department of Community Health
Jennifer M. Granholm, Governor
Janet Olszewski, Director

LabLink

Michigan Department of Community Health
Bureau of Laboratories

Vol. 9 No. 1 **Fall 2003**

Pertussis in Michigan

James Rudrik, Ph.D.
Microbiology Section

Despite the availability of an effective vaccine, pertussis cases have continued to increase in the United States. These increases first noted in the 1980s have occurred primarily in infants too young to receive the vaccine and in adolescents and adults. A total of 29,134 cases of pertussis were reported to the Centers for Disease Control and Prevention (CDC) between 1997 and 2000. Compared with data from 1994 to 1996, the incidence of pertussis increased 62% and 60% for adolescents and adults, respectively. The rate in infants increased 11%, while the rate in children aged 1-4 decreased 8% and the rate in children 5-9 years remained stable (2).

In Michigan, 715 cases of pertussis were reported between 1995 and 2002 with a peak of 149 cases reported in 2001. Fifty-six percent of cases occurred in children under 2 years of age. Between 1995 and 1998, 19 cases were reported in adolescents age 11 to 19 and 19 cases were reported in adults. During the 1999 to 2002 timeframe, 35 cases occurred in adolescents and 62 cases occurred in adults.

The increase in the reported incidence of pertussis may be due to improved diagnostic tests such as PCR, or to increased recognition of pertussis as a diagnosis in older age groups. However, the increase incidence in infants, a well-recognized, high-risk group, suggests a true increase in pertussis. Some studies have also suggested that *Bordetella pertussis* has adapted to remain endemic despite widespread vaccination (3).

Accurate laboratory identification of *B. pertussis* infections has been problematic. While culture was considered the "gold standard" because of specificity, its sensitivity was markedly influenced by factors like antibiotic treatment, duration of symptoms prior to specimen collection and specimen transport conditions. Direct fluorescent antibody (DFA) testing provides a rapid diagnosis, but suffers from poor sensitivity and variable specificity. Serology is frequently most useful to confirm a diagnosis after the fact. In the mid-1990s, many laboratories began using PCR to detect *B. pertussis* DNA in clinical specimens. Laboratory diagnosis of pertussis now includes either a positive culture or positive PCR result for *B. pertussis* (1).

PCR offers the advantages of being sensitive, specific and provides a rapid turn around time. The main disadvantage to PCR is that it fails to provide a viable organism for additional testing. Isolates are important for molecular epidemiology and detecting phenotypic strain markers for outbreak investigations. Several studies have shown that PCR is significantly more sensitive than culture and that 2 to 51% of PCR positive specimens are culture negative (4). Several different regions of the *B. pertussis* genome have been used as targets for amplification, including the pertussis toxin gene, the porin gene, and the adenylate cyclase gene and insertion sequence IS *481*. Assays utilizing IS *481* as the target appear to be more sensitive based on copy number. There are 50 to 100 copies of IS *481* in

the *B. pertussis* chromosome compared to a single copy of the pertussis toxin gene (5).
MDCH has offered both culture and PCR for *B. pertussis* for several years, but they were performed on different specimens. The culture was performed from a nasopharyngeal (NP) swab and PCR was performed only from an NP wash or aspirate. An NP swab in an Amies charcoal transport medium proved to be a poor sample for PCR and an NP wash without a transport medium failed to yield viable organisms when cultured. As a result, the laboratory received relatively few requests for PCR. The laboratory recently validated a PCR method for NP swabs transported in Regan-Lowe medium. The use of Regan-Lowe does not interfere with PCR and still provides an excellent specimen for culture. PCR and culture are now performed on all appropriately collected specimens. PCR results are available within 48 hours of specimen receipt, while final culture results require two weeks.

The PCR assay utilizes IS *481* as the target. While this target provides excellent sensitivity, the insertion sequence can also be found in *Bordetella holmesii.* This organism was first described in 1995 and has been associated with septicemia, endocarditis, and respiratory symptoms (6). *B. holmesii* can be found in respiratory swab specimens, but the organism fails to grow on most media used to isolate *Bordetella* spp. because cephalexin in these media inhibits this organism. A recent study showed that *B. holmesii* was isolated from 0.3% of NP cultures from patients suspected of having pertussis; *B. pertussis* was cultured from 7% (7). The role of *B. holmesii* in cough-associated illness and its prevalence in asymptomatic individuals remains to be elucidated.

Specimen collection Unit 15 available from MDCH contains an NP swab, Regan-Lowe medium, a test requisition, and directions for specimen collection and shipping. To obtain maximum sensitivity for both PCR and culture, the NP swab should be inserted into the Regan-Lowe transport medium and incubated for 18 to 24 hours at 35° C prior to shipment by the most expeditious method. Specimen collection kits may be obtained by contacting the laboratory support unit (phone 517-335-9867, fax 517-335-9039, e-mail DietzR@michigan.gov.) The laboratory support unit is opened 8:00 a.m. until 4:30 p.m. Monday through Friday.

References:

1. Centers for Disease Control and Prevention. 1997. Case definitions for infectious conditions under public health surveillance. MMWR 46(RR-10): 25.

2. Centers for Disease Control and Prevention. 2002. Pertussis - United States, 1997 – 2000. MMWR 51(04): 73-76.

3. Mooi, F.R., I.H.M. vanLoo, and A.J. King. 2001. Adaptation of *Bordetella pertussis* to vaccination: a cause for reemergence? Emerg. Infect. Dis. 7 (No. 3 suppl.): 526-528.

4. Muller, F.M., J.E. Hoppe, and C.H. Wirsing von Konig. 1997. Laboratory diagnosis of pertussis: State of the art in 1997. J. Clin. Microbiol. 35:2435-2443.

5. Sloan, L.M., M.K. Hopkins, P.S. Mitchell, E.A. Vetter, J.E. Rosenblatt, W.S. Harmsen, F.R. Cockerill, and R. Patel. 2002. Multiplex LightCycler PCR assay for detection and differentiation of *Bordetella pertussis* and *Bordetella parapertussis* in nasopharyngeal specimens. J. Clin. Microbiol. 40:96-100.

6. Weyant, R., D. Hollis, R. Weaver, M. Amin, A. Steigerwalt, S. O'Conner, A. Whitney, M. Daneshvar, C. Moss, and D. Brenner. 1995. *Bordetella holmesii* sp. nov., a new gram-negative species associated with septicemia. J. Clin. Microbiol. 33:1-7.

7. Yih, W., E. Silva, J. Ida, N. Harrington, S. Lett, and H. George. 1999. *Bordetella holmesii*-like organisms isolated from Massachusetts patients with pertussis-like symptoms. Emerg. Infect. Dis. 5:441-443.

Congratulations!!!!

Dedication to education while working in a full-time position is a trait to be recognized. The MDCH Bureau of Laboratories would like to congratulate Carrie Anglewicz and Barbara Evans. Both have received a Molecular Biology Certificate. This certification was earned through the Medical Technology program at Michigan State University. Anglewicz and Evans are both microbiologists in the Microbiology section.

Locally-Acquired Quinolone-Resistant *Neisseria gonorrhoeae* in Michigan

Dara Ganoczy, MPH
Bureau of Epidemiology

Several areas of the country are seeing increases in reported cases of quinolone-resistant gonorrhea (QRNG). As of September 30, 12 cases of QRNG had been identified in Michigan this year. Seven were Ingham County residents; three were from Kent County, and one each in Washtenaw and Wayne counties. Ten cases were male, of which three were men who had sex with men (MSM), and ages ranged from 16 to 42 years. Six of the cases were Black, four were White, and one was Asian and one Hispanic. Only two cases (Washtenaw and Kent counties) reported travel to areas with endemic QRNG; the others appear to have acquired their infections locally. Michigan has had one or two QRNG cases each year, all reporting recent travel to Asia.

Following identification of the first QRNG cases in Ingham County, the Sexually Transmitted Disease (STD) program notified all local health departments of changes in treatment recommendations for patients in Ingham and adjoining counties (Clinton, Eaton, Jackson, Livingston and Shiawassee). Patients in these counties are to receive a non-quinolone regimen for the treatment of gonorrhea. Providers in other counties are advised to obtain a travel history for patients being treated for gonorrhea. If they had sex partners from Asia, Hawaii, California, Ingham or adjoining counties, they too should be treated with a non-quinolone regimen. The Kent County Health Department issued an advisory recommending that providers culture all MSM being evaluated for gonorrhea and submit cultures to the state lab in Lansing for susceptibility testing. No changes in treatment of MSM in Kent County are recommended at this time. Providers in all counties are encouraged to obtain specimens for culture from patients whose symptoms do not resolve and send isolates to the state lab in Lansing for susceptibility testing.

The Centers for Disease Control and Prevention (CDC) recommended in the 2002 STD Treatment Guidelines that states track gonococcal resistance to assist providers in prescribing the most effective antibiotics for their patients. The Michigan Department of Community Health has been conducting a gonococcal resistance surveillance project in conjunction with sentinel labs around the state since July 2002. In addition, the Detroit STD Clinic joined the national Gonococcal Isolate Surveillance Project in January 2003. MDCH will continue to monitor reports of QRNG. As additional cases are detected, treatment guidelines may be revised. For more information regarding culture submission, contact Dr. Jim Rudrik, manager of the microbiology section, at 517-335-9641or at rudrikj@michigan.gov.

Notes on Printing Photo Micrographs

William Sottile, PhD, ABMM
Regional Laboratory Coordinator

Printing graphic images from a computer is simple. Getting a good quality print is not so simple. Several factors need to be addressed to obtain a high quality print. Consider the nature and quality of the image; it's format, size and resolution. Even a high quality image with good contrast, brightness and color, you will not be able to produce a 'photo quality' print unless you have the appropriate printer.

Most of the images stored are in ***.jpg** or ***.bmp** format. The jpg format allows for significant compression of a large image without too much loss of detail. However, every time a jpg image is opened, modified and saved it will loose some of its clarity, like a copy of a copy of a copy. Images with bmp format take up much more space on a hard drive than jpg, but they can be opened and saved many times with no loss of image quality.

To obtain a quality printed output (photo) a printer that is capable of producing photo quality output is needed. Most inkjets with resolutions in excess of 1200 x 600 dpi will do the job. Higher resolution is better. The size of the ink droplet (smaller the better) and the number of inks used (the more the better) has a significant effect on the quality of image. MDCH uses an HP 1100 inkjet with a resolution of 2400 x 1200 dpi or 600 x 600. Most laser jet printers only produce 300 x 300 or 600 x 300 in draft and high quality mode respectively, which will not produce a particularly sharp picture. Networked copier/printers are limited to 300 x 300 dpi and produce very mediocre photographs. Laser jets will give you an idea of what is in a picture, but it will not show the level of detail and gradation in shades that a higher quality image will yield.

Paper and ink have a surprising effect on the quality of output. A fair print can be produced using bright copy paper and generic ink, but photo quality paper designed for inkjet printing should be used for higher quality prints. Price and quality are directly related. Use ink and paper designed specifically for your brand of printer. Substitutes may work but significant time may be spent trying to find a combination that works well. When ready to print, it is important to select the "properties" or "advanced" button in the printer dialog box and specify paper type and the resolution. Print a single page with only one photo until the output is correct. Then produce one copy of the primary document. Once the output achieves the quality desired, print the number of copies needed.

Laboratory Response Network Tracks Resistant GC

Martha Boehme, MT(ASCP)
Division of Infectious Diseases

As referred to in the article by Dara Ganoczy (page 3), since July 2002, MDCH has been conducting a gonococcal resistance surveillance project in conjunction with select sentinel labs around the state. The results of that surveillance reveal that quinolone-resistant *Neisseria gonorrhoeae* (QRNG) is making steady progress into Michigan. What the results do not readily reveal, however, is the contribution of five sentinel laboratories that have been faithfully submitting isolates for the study.

Early in 2002, hospital and local health department laboratories were contacted to determine their gonococcal (GC) testing practices. Since a majority of laboratories have switched to non-culture methods for GC testing, finding laboratories both able and willing to provide isolates was a substantial hurdle. Fortunately, one laboratory was already sending isolates to MDCH routinely and an additional four laboratories agreed to submit isolates to MDCH lab for routine susceptibility testing for a period of six months.

The mathematicians in the reading audience have already figured out that this is the longest six-month surveillance on record, as it has continued for fifteen months now. At the request of the Centers for Disease Control and Prevention, the culture surveillance has also been expanded to additional STD clinics. These clinics and microbiology laboratories are expending considerable effort to provide MDCH with GC isolates. MDCH would like to acknowledge their dedication.

Keeping GC isolates viable for transport is no small task. Laboratories either subculture the isolates every other day or freeze them until they can be inoculated onto chocolate agar slants for shipment. Packaging the slants to comply with infectious substance regulations, mindful of the MDCH GC laboratory Monday through Friday testing schedule, takes both time and dedication. In spite of these difficulties, the majority of these fastidious isolates arrive in viable condition. After identification and susceptibility testing are performed in the microbiology section, resistant isolates are submitted to the molecular biology section where developing technology is used to detect resistance genes by genetic sequencing. Finally, cultures are sent to the CDC for further characterization.

The spread of any antimicrobial resistance has sobering implications, but QRNG brings with it some distinct social and economic ramifications. Fluoroquinolones are preferred by patients (oral) and healthcare (cheaper). Its manufacturer withdrew Cefixime, the only other oral recommended drug, from the market in 2002. Ceftriaxone and cefixime minimum inhibitory concentrations, although still in the susceptible range, are creeping upward. There is speculation that it may be only a matter of time before these agents become ineffective. Therefore, changing treatment recommendations is a serious step that requires solid laboratory data. MDCH is fortunate to have clinical laboratory partners who understand the importance of their contribution to these major developments and is very grateful to these microbiology laboratories, without whose participation the QRNG might have gone undetected for some time. Also commended are the staff in the Data and Specimen Handling (DASH) unit, Quality Control unit, and the Enterics/STD/Chromatography unit who have adjusted workflow practices to accommodate the extra testing and who provide the support needed to carry on a project of this scope.

The strength of the laboratory response network in Michigan depends upon the foundation provided by the sentinel clinical laboratories. They are vital partners in protecting and improving the health of the citizens of Michigan.

Prevention of Perinatal Group B Streptococcal Disease

Patricia Somsel, Dr. P.H.
Division of Infectious Diseases

In 1996, the Centers for Disease Control and Prevention (CDC) issued the first guidelines on prevention of perinatal Group B streptococcus (GBS). These guidelines recommend the use of one of two prevention methods, one based upon identification of intrapartum risk factors and the other on a culture-based screening approach. The culture-based screen recommends culturing all pregnant females for vaginal and rectal GBS colonization between 35 and 37 weeks' gestation.

Before these guidelines were promulgated, incidence of early and late onset GBS neonatal disease occurred at a rate of 1.7 cases per 1,000 live births in 1993. Earlier estimates suggested a rate of 2-3 cases per 1,000 live births or an estimated 7,500 cases per year. After the introduction of the initial guidelines, the incidence of GBS neonatal disease fell 65% from the 1993 rate to 0.5 cases in 1999, preventing an estimated 4,500 cases and 225 deaths in the latter year. There appears to also have been

an associated decline in invasive maternal infections from 0.29 per 1,000 live births in 1993 to 0.23 in 1998, a 21% decrease.

The incidence of late-onset GBS disease appears to have remained steady despite use of intrapartum antibiotics to prophylax maternal colonization or address intrapartum risk factors, suggesting that these interventions are ineffective against late-onset disease. GBS still causes an estimated 1,600 early-onset cases and 80 deaths annually. Analysis of 1996 data comparing the efficacy of risk-based versus culture-based screening in preventing disease provided clear evidence that the latter was more effective in preventing GBS disease. In 18% of births, women did not have any risk factors to prompt antibiotic prophylaxis yet were culture positive. These findings prompted the development of updated guidelines that may prove challenging to clinical microbiology laboratories as well as to clinicians.

The updated guidelines were published in August of 2002 [MMWR 2002;51 (RR-11)] and called for universal culture-based screening of all pregnant women. A vaginal/rectal specimen is to be cultured in selective broth unless a woman had GBS bacteriuria during the current pregnancy or previously had an infant with invasive GBS disease. An informal and nonscientific poll taken of clinical microbiologists in Michigan indicates a number of problems are being encountered in meeting these guidelines.

The presence of GBS in a urine culture from a pregnant woman has been identified as a marker for heavy genital colonization and should prompt intrapartum prophylaxis. The experience of most clinical microbiologists suggests obtaining any information on a patient beyond age and gender is a substantial challenge, especially with outpatients. Some laboratory information systems (LIS) do not readily accept or transfer such information to the microbiologists plating and reading the culture, if indeed the information is provided on the test request or the specimen container. A number of laboratories, in an effort to meet the guidelines, evaluate all urine cultures from women in their childbearing years for the presence of GBS and the report the findings. One consequence of this may be the unnecessary use of antibiotics in women who are not pregnant by clinicians who lack sufficient understanding of the complications of the laboratory component of the algorithm.

When small numbers of GBS are contaminants in a clean-voided urine, it may be very difficult to recognize them. Up to 11% of GBS colonies will lack the characteristic beta hemolysis, making their differentiation from the enterococci problematic. In cultures in which nonhemolytic GBS predominates, the presence of other contaminants may hinder recognition of GBS.

According to the guidelines, GBS isolated from a woman allergic to penicillin should be tested for susceptibility to clindamycin and erythromycin if the woman is allergic to penicillin. Again the issue of communicating relevant patient information is an obstacle. Laboratories may find it difficult to economically justify testing all GBS recovered from vaginal/rectal cultures when specific knowledge of penicillin allergy is lacking. Some laboratories do not routinely perform susceptibility testing on GBS isolates and may lack the necessary reagents and materials, necessitating referral to another laboratory. The expense incurred for this 2-disc test may not be reimbursed as it uses a non-standard billing code. Some laboratories hold the organism for a week and await orders for a susceptibility test while the physician is notified of the culture results. This delays results for a woman who may be approaching term. These susceptibility-testing issues have particular relevance in communities of Michigan that have elevated levels of resistance to clindamycin.

Full implementation of the 2002 guideline recommendations for clinical microbiology laboratories clearly requires careful thought and communication with clinicians about the essential role of information sharing to assure appropriate testing.

The following quote concludes the CDC guidelines: "Before full implementation of this strategy can be expected in all health-care settings, all members of the health-care team will need to improve protocols for isolation and reporting of GBS culture results, to improve information management to ensure communication of screening results, and to educate medical and nursing staff responsible for prenatal and intrapartum care. Within institutions, such efforts may take several months."

It is a year beyond the publication of these guidelines and it appears from this unscientific poll that it will require a determined effort by clinical microbiology laboratories in Michigan and their medical communities to fully implement them.

The MDCH Bureau of Laboratories web page can now be found at:

www.michigan.gov/mdchlab

Bookmark this site and use it often.

FUN FUNGI.....

Differentiating *Microsporum canis* From *Microsporum gypseum*.

Sandy Arduin MT(ASCP) & Bruce Palma MT(ASCP) - Mycobacteriology/Mycology Unit

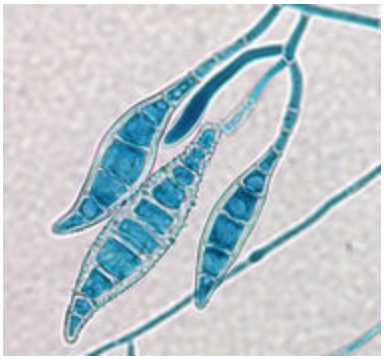
Microsporum canis

Microsporum canis is a dermatophyte that causes infection of the scalp and glaborous skin (trunk) in humans. It is often a cause of ringworm infection in animals (cats, dogs, monkeys). Growth on artifical media is rapid and appears downy to wooly. Colonies have a white to cream or pale yellow surface and a yellow to yellow-orange on the reverse side. Microscopically, microconidia are clavate (club shaped) and rarely seen. Typically, only macroconidia are present. Macroconidia are fusoid (spindle shaped) with a thick, sometimes verrucose (rough) or echinulate (spiny) cell wall. The macroconidia have a recurved apex (bent tip) and contain 5-15 cells. *M. canis* is hair perforation test positive.

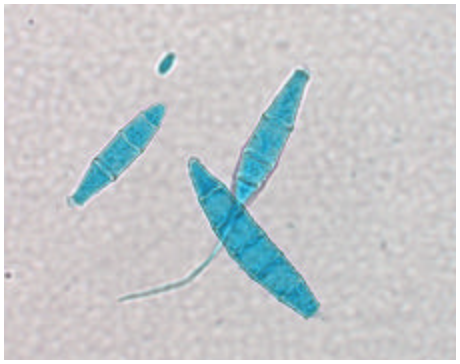
Microsporum gypseum

Microsporum gypseum is frequently isolated from the soil and the fur of small rodents. It occasionally causes infection of the scalp and glaborous skin (trunk) in humans. Growth on laboratory media is rapid, downy, and becomes powdery to granular. Colonies are cream, tawny-buff, or pale cinnamon colored with a beige to red brown reverse side. Microscopically, microconidia are moderately abundant and club shaped. Macroconidia are abundant, ellipsoidal to fusiform, sometimes verrucose, and thin walled. The macroconidia typically contain 3-6 cells. *M. gypseum* is hair perforation test positive.

Microsporum canis



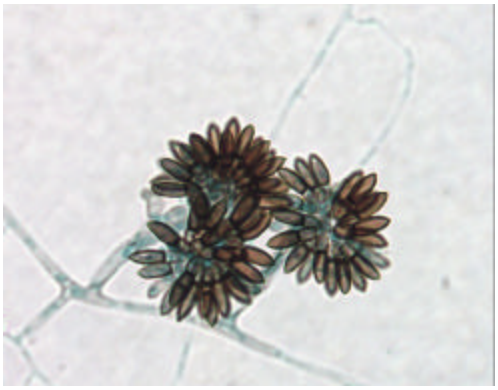
Microsporum gypseum



Differentiating *Microsporum canis* from *Microsporum gypseum*

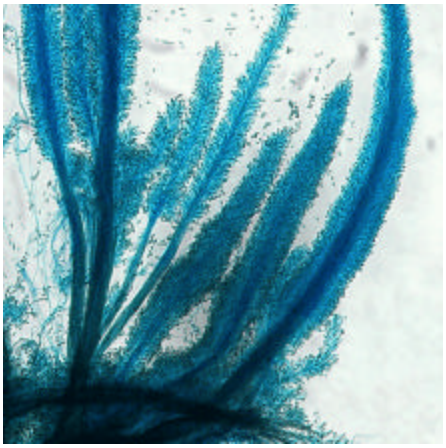
	M. canis	***M. gypseum***
Color	Cream to yellow/ yellow-orange reverse	Tawny-buff to cinnamon/ red brown reverse
Texture	Downy	Powdery to granular
Cell wall	Thick walled with recurved apex, 5-15 cells.	Thin walled, 3-6 cells
Woods lamp	Fluorescent	Little or no fluorescence

Last Issue's Picture Quiz Answer:

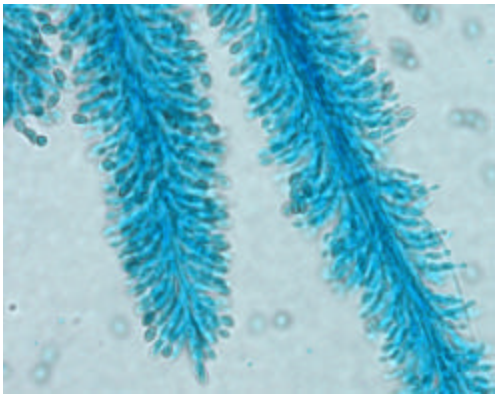


This picture was of *Wardomyces* spp., which is a relatively uncommon soil fungus. The colony was pink/grey, wrinkled and powdery. The hyphae were hyaline and the conidiophores were branched. The sporogenous cells bore apical clusters of dark ellipsoidal conidia. The conidiogenous cells were globose to barrel shaped and produced several conidia. The conidia were generally 2-celled and constricted at the septum. The apical cell was twice as long as the basal cell.

This Issue=s Picture Quiz: What Mould is this?



20X



100X

This mould was received as a referred culture from a bronchial specimen. The colony was white and velvety. Microscopically, dematiaceous conidiophores formed a synnemata with a fertile spore bearing head. The head was elongate and feathery, comprised of a central axis of hyphae, which bore numerous annellophores. The anellophores were short and inflated and sometimes produced long chains of spores.

Recent Publications from MDCH Employees

Transmission of West Nile Virus through Blood Transfusion in the United States in 2002

N Engl J Med 2003;349:1236-45.

Lisa N. Pealer, Ph.D., Anthony A. Marfin, M.D., M.P.H., Lyle R. Petersen, M.D., M.P.H., Robert S. Lanciotti, Ph.D., Peter L. Page, M.D., Susan L. Stramer, Ph.D., Mary Grace Stobierski, D.V.M., M.P.H., Kimberly Signs, D.V.M., Bruce Newman, M.D., **Hema Kapoor, M.D.**, Jesse L. Goodman, M.D., M.P.H., and Mary E. Chamberland, M.D., M.P.H., for the West Nile Virus Transmission Investigation Team

Severe Morbidity and Mortality Associated with Influenza in Children and Young Adults -- Michigan, 2003

MMWR September 5, 2003 / 52(35);837-840-840

Reported by: MJ Wilkins, DVM, ML Boulton, MD, GA Stoltman, PhD, SA Bidol, MPH, KS Enger, MPH, JJ Lai, MPH, Michigan Dept of Community Health. T Uyeki, MD, S Harper, MD, Div of Viral and Rickettsial Diseases; M Fischer, MD, SP Reagan, MPH, Div of Bacterial and Mycotic Diseases; J Jones, MD, P Terebuh, MD, SD Stonecipher, DVM, EIS officers, CDC.

LabLink is published quarterly by the Michigan Department of Community Health, Bureau of Laboratories, to provide laboratory information to Michigan health professionals and the public health community.

Director, Bureau of Laboratories
Frances Pouch Downes, Dr.P.H.

Editor
Susan L. Shiflett